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(54) Title: SYNTHETIC ANTIGENS FOR THE DETECTION OF AIDS-RELATED DISEASE

(57) Abstract

Novel peptides are provided having substantially the same sequence as immunologically significant fragments of AIDS-related viruses. The polypeptides can be used as reagents in the determination of exposure of a human host to the virus. Of particular interest is the use of polypeptides in screening blood products.

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SYNTHETIC ANTIGENS FOR THE DETECTION OF AIDS-RELATED DISEASE

BACKGROUND OF THE INVENTION

Field of the Invention

With the discovery that the diseases called lymphadenopathy syndrome and acquired immune deficiency disease (AIDS) are caused by an infectious retrovirus designated lymphadenopathy virus (LAV), human T-cell lymphotropic virus-III (HTLV-III), AIDS-related virus (ARV), or immune deficiency-associated virus (IDAV), there has become an immediate need to be able to detect potential vectors of the disease, such as blood from diseased individuals, which may be employed for transfusions or from which specific blood factors may be isolated.

To detect potential vectors of the disease, it is necessary to have viral proteins and/or antibodies to such proteins. Because of the hazards associated with growing the LAV/HTLV-III retrovirus, there is significant interest in establishing means for obtaining the viral proteins or their immunologic equivalents, which means do not necessitate handling large volumes of live, potentially infectious virus. In choosing alternatives, one must be concerned with the fact that the viruses have been reported to be highly polymorphic, frequently changing as the retrovirus is passaged.

Brief Description of the Relevant Literature

The various antigens of the retrovirus are described by Saxinger et al., Science (1985) 227:1036-1038. See also Gallo et al., ibid. (1984) 224:500; Sarangadharn et al., ibid. 224:506; Barre-Sinoussi et al., ibid. (1983) 220:868; Montagni r et al., in Human T-Cell Leuk mia/Lymphoma Virus, Gallo, Essex, Gross, eds. (Cold Spring Harbor Laboratory, Cold

Spring Harbor, N w York), 1984, p. 363. These may includ , but are not limited to, p13, p18, p25, p36, gp43, p55, gp65, gp110, etc., where the numbers may differ depending upon the reporter.

Hopp and Woods, Proc. Natl. Acad. Sci. USA (1981) 78:3824, describe criteria for selecting peptides as potential epitopes of polypeptides based on their relative hydrophilicity. In one study employing these criteria, a 12-amino acid peptide was synthesized that bound 9% of antibodies elicited by the native protein (Hopp, Molec. Immunol. (1981) 18:869). In general, Hopp/Woods criteria have been shown not to have a high predictive value. Furthermore, epitopes have been demonstrated which are not hydrophilic (Kazim et al., Biochem. J. (1982) 203:201). Other studies of polypeptide antigenicity include Green et al., Cell (1982) 28:477, where peptides were employed which elicited antibodies, which antibodies were capable of binding to the native protein, while conversely antibodies which were elicited by the native protein failed to bind to the peptides; and Trainer et al., Nature (1984) 312:127, whose results with myohaemerythrin paralleled those of Green et al.

The complete nucleotide sequence of LAV is reported by Wain-Hobson et al., Cell (1985) 40:9. The complete sequence for HTLV-III is reported by Muesing et al., Nature (1985) 313:450, while the complete sequence for ARV is reported by Sanchez-Pescador et al., Science (1985) 227:484. All three viruses exhibit substantial nucleotide homology and are similar with respect to morphology, cytopathology, requirements for optimum reverse transcriptase activity, and at least some antigenic properties (Levy et al., Science (1984) 225:840; Shupbach et al., Science (1984) 224:503), and hence should be consider d isolates of the same virus. See also, Chang et al., Science (1985) 228:93.

SUMMARY OF THE INVENTION

Peptide sequences capable of immunologically mimicking proteins encoded in the gag and/or env regions of the LAV/HTLV-III retrovirus are provided as reagents for use in the screening of blood and blood products for prior exposure to the retrovirus. The peptides are of at least 5 amino acids and can be used in various specific binding assays for the detection of antibodies to LAV/HTLV-III virus, for the detection of LAV/HTLV-III antigens, or as immunogens.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

For the purpose of this disclosure, a virus is considered to be the same as or equivalent to LAV/HTLV-III if it substantially fulfills the following criteria:

(a) The virus is tropic for T-lymphocytes, especially T-helper cells ($CD4^+$, according to the international nomenclature defined in Bernard et al., eds. Leucocyte Typing, New York: Springer Verlag, 1984);

(b) The virus is cytopathic for infected $CD4^+$ cells (rather than transforming, as are HTLV-I and -II);

(c) The virus encodes an RNA-dependent DNA polymerase (reverse transcriptase) which is Mg^{2+}-dependent (optimum concentration 5mM), has a pH optimum of 7.8, is not inhibitable by actinomycin D, and can employ oligo$(dT)_{12-18}$ as a primer for reverse transcription from its 3' LTR;

(d) The virus bands in a sucrose gradient at a density of approximately 1.16;

(e) The virus can be labeled with $[^3H]$-uridine;

(f) The virus is substantially cross-reactive immunologically with th proteins encoded by th gag and env regions of LAV/HTLV-III; and

(g) The virus shares substantial nucleotide homology (approximately 75-100%) and amino acid sequence homology (approximately 75-100%) with LAV or HTLV-III.

Novel peptides are provided which immunologically mimic proteins encoded by the LAV/HTLV-III retrovirus, particularly proteins encoded by the gag and/or env regions of the viral genome. To accommodate strain-to-strain variations among different isolates, adjustments for conservative substitutions and selection among the alternatives where non-conservative substitutions are involved, may be made. These peptides can be used individually or together for detection of the virus or of antibodies to the virus in a physiological sample. Depending upon the nature of the test protocol, the peptides may be labeled or unlabeled, bound to a solid surface, conjugated to a carrier or other compounds, or the like.

The peptides of interest will be derived from the peptides encoded by the gag region or the env region. These peptides will be primarily derived from p55 or fragments thereof, e.g., p25 and p18, or gp150 and fragments thereof, e.g., gp41. These peptides will be given Roman numerals, but will also be given numerical designations which are arbitrarily associated with the manner in which they were produced.

For the gag region, of particular interest are the coding regions extending from about base pair (bp) 450 to bp 731, particularly from about bp 450 to bp 545 (97) and bp 696 to bp 731 (71); from about bp 900 to bp 1421, particularly from about bp 921 to bp 1016, including bp 921 to bp 1010; bp 972 to bp 1016 (92); and bp 936 to bp 995 (17); or from about bp 1158 to about bp 1400, particularly bp 1164 to bp 1250 (90); bp 1278 to bp 1385 (88); and bp 1320 to bp 1385 (15), of the LAV/HTLV-III retrovirus. (Numbering according to Wain-Hobson et al., supra.)

For the env region, the regions of particular interest will be those polypeptides encoded within the bp 7210 to bp 7815 regions, particularly within bp 7231 to bp 7794, more particularly within about bp 7246 through bp 7317 (36), bp 7516 through bp 7593 (39), particularly bp 7543 through bp 7593 (79) and bp 7561 through 7593 (78), bp 7708 through bp 7779 (23), bp 7630 through bp 7689 (40), bp 7498 through bp 7554 (56).

The peptides of interest will include at least five, sometimes six, sometimes eight, sometimes 12, usually fewer than about 50, more usually fewer than about 35, and preferably fewer than about 25 amino acids included within a sequence coded for by the LAV/HTLV-III retrovirus. In each instance, desirably the oligopeptide will be as small as possible, while still maintaining substantially all of the sensitivity of the larger peptide. In some instances it may be desirable to join two or more oligopeptides which are non-overlapping in the same peptide structure or as individual peptides, which separately or together provide equivalent sensitivity to the parent.

The peptides may be modified by introducing conservative or non-conservative substitutions in the peptides, usually fewer than 20 number percent, more usually fewer than 10 number percent of the peptides being exchanged. In those situations where regions are found to be polymorphic, it may be desirable to vary one or more particular amino acids to more effectively mimic the differing epitopes of the different retroviral strains. In many instances to provide chemical and physical stability, methionine may be replaced by norleucine (Nor).

It should be understood that the polypeptides employed in the subject invention need not be identical to any particular LAV/HTLV-III polypeptide sequence, so long as the subject compounds are able to provid for immunological competition with proteins of at least one of the strains of th LAV/HTLV-III retrovirus.

Th refore, the subject polypeptides may be subject to various changes, such as insertions, deletions, and substitutions, either conservative or non-conservative, where such changes might provide for certain advantages in their use. By conservative substitutions is intended combinations such as gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg; and phe, tyr. Usually, the sequence will not differ by more than twenty percent from the sequence of at least one strain of an LAV/HTLV-III retrovirus except where additional amino acids may be added at either terminus for the purpose of providing an "arm" by which the peptides of this invention may be conveniently immobilized. The arms will usually be at least 1 amino acid and may be 50 or more amino acids, more often 1 to 10 amino acids.

In addition, one or two amino acids may be added to the termini of an oligopeptide or peptide to provide for ease of linking of peptides one to another, for coupling to a support or larger peptide, for reasons to be discussed subsequently, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like.

Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, may be introduced at the C- or N-terminus of the peptide or oligopeptide to provide for a useful functionality for linking. Of particular interest is the presence of from 1 to 3 cysteines at the C- or N-terminus for linking to a support. The cysteine could be linked through a disulfide linkage to a dithio- or thio-functionalized support on a thioether linkage to an activated olefin support.

In addition, the peptide or oligopeptide sequences may differ from the natural sequence by the sequence being modified by terminal-NH_2 acylation, .g., acetylation, or thioglycolic acid amidation, terminal-carboxy amidation, e.g., ammonia, methylamine, etc. In some instances, these modifications may

provide sites for linking to a support or other molecule.

The peptides and oligopeptides of interest will now be considered. The first peptides of interest will be derived from the gag region, particularly the protein referred to as p25 and p18.

The peptides for p25 are as follows:

The peptide I (15) encoded in the region bp 1320 to bp 1385 will have the following amino acid sequence, where oligopeptides included within the following sequence will include linear epitopes within such sequence:

(I) (15)

Y-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Met-Met-Thr-Ala-Cys-X, where X is OH or NH_2,

wherein the amino terminal Y, e.g., Tyr or Cys, if present, is added to facilitate coupling of the peptide to a protein carrier.

The next peptide II (17) will be encoded by the region extending from about bp 936 to bp 995 and will have the following sequence, where oligopeptides included within the following sequence will include linear epitopes within such sequence:

(II) (17)

Y-Leu-Lys-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg-Val-His-Pro-Val-His-Ala-X, where X is OH or NH_2,

wherein the amino terminal Y has been defined previously.

Of particular interest is th oligopeptide IIa:

(IIa)

Y-Ala-Ala-Glu-Trp-Asp-Arg-Z-X,

wherein X and Y have been defined previously and Z is a bond, an amino acid which provides a means of linking, e.g., cysteine, tyrosine, etc., or taken together with X provides a functional group which may be used for linking, e.g., an olefin as in allyl or maleimidyl, dithio, etc.

The next peptide of interest, III (92), will be encoded by the region extending from about bp 972 to bp 1016 and will have the following sequence, where oligopeptides included within the following sequence will include linear epitopes within such sequence:

(III) (92)

Y-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile-Ala-Pro-Gly-Gln-X,

wherein X, Y and Z have been defined previously.

Preferably, this peptide will have no more than about 15 amino acids encoded by the LAV/HTLV III genome.

The next peptide, IV (90), will be encoded by the region extending from about bp 1164 to bp 1250 and will have the following sequence, where oligopeptides included within the following sequence will include linear epitopes within such sequence:

(IV) (90)

Y-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-Asp-Ile-Arg-Gln-Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-Asp-Arg-Phe-Tyr-Lys-Thr-Leu-Arg-Z-X,

wherein X, Y and Z hav been defined previously.

Preferably, this peptide will have no more than about 29 amino acids encoded by the LAV/HTLV III genome.

The peptide, V (88), will be encoded by the region xtending from about bp 1278 to bp 1385 and will have the following sequence, where oligopeptides included within the following sequence will include linear epitopes within such sequence:

(V) (88)

Y-Asn-Trp-Nor-Thr-Glu-Thr-Leu-Leu-Val-Gln-Asn-Ala-Asn-Pro-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Nor-Nor-Thr-Ala-Cys-X,

wherein X and Y have been defined previously.

The next peptides of interest will be derived from the gag protein region referred to as p18.

The next peptide of interest, VI (97), will be encoded by the region extending from about bp 450 through bp 545 and will have the following sequence, where oligopeptides included within the following sequence will include linear epitopes within such sequence:

(VI) (97)

Y-Arg-Glu-Leu-Glu-Arg-Phe-Ala-Val-Asn-Pro-Gly-Leu-Leu-Glu-Thr-Ser-Glu-Gly-Cys-Arg-Gln-Ile-Leu-Gly-Gln-Leu-Gln-Pro-Ser-Leu-Gln-Thr-X,

wherein X and Y have been defined previously.

The next peptide of interest VII (71) will be encoded by the region extending from about bp 696 to bp 731. This peptide will include any oligopeptides coding for linear epitopes with the following amino acid sequence:

(VII) (71)

Y-Asp-Thr-Gly-His-Ser-S r-Gln-Val-S r-Gln-Asn-Tyr,

wherein Y has been defin d previously.

The next polypeptides of interest will be those derived from the env region, from gp110 (110kDal).

The next peptide of interest, VIII, will be encoded by the region extending from about bp 7246 through bp 7317 and while coming within the general limitations indicated previously, will preferably have no more than 24 amino acids encoded by the LAV/HTLV III genome.

The peptide of interest will generally have the following amino acid sequence, where oligopeptides included within the following sequence will include linear epitopes within such sequence:

(VIII) (36)

Val-Lys-Ile-Glu-Pro-Leu-Gly-Val-Ala-Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Ala-Z-X, where X is OH or NH_2,

wherein the carboxy terminal Z, e.g., Cys, if present, is an amino acid added to facilitate coupling of the peptide to a protein carrier.

Of particular interest is where 6, conveniently up to 4, of the naturally occurring C-terminal amino acids are deleted or substituted.

Oligopeptides contained within the above sequence of particular interest include:

(VIIIa) (49)

Y-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Z-X

(VIIIb) (50)

Y-Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-X.

Th next peptides of interest will be derived from the env region known as gp41.

The next peptide, IX (56), will be encoded by the region extending from about bp 7498 to bp 7554, where oligopeptides included within the following sequence will include linear epitopes within such sequence:

(IX) (56)

Ile-Lys-Gln-Leu-Gln-Ala-Arg-Ile-Leu-
Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Z-X,

wherein X, Y and Z have been defined previously.

Oligopeptides contained within the above sequence of particular interest include:

(IXa) (56/39)

Y-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-
Lys-Asp-Gln-Gln-Z-X

and

(IXb) (39/56)

Y-Ile-Lys-Gln-Leu-Gln-Ala-Arg-Ile-Leu-Z-X.

The next peptide of interest, X (39), will be encoded by the region from about bp 7516 through bp 7593 and has the following amino acid sequence, where oligopeptides included within the following sequence will include linear epitopes within such sequence:

(X) (39)

Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-
Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-
Ser-Gly-Lys-Leu-Ile-Cys-X, where X is OH or NH_2.

The next peptide, XI (40), will b encod d by the region extending from about bp 7630 to 7689 where

oligop ptides included within the following sequence will include linear epitopes within such sequence:

(XI) (40)

Y-Lys-Ser-Leu-Glu-Gln-Ile-Trp-Asn-Asn-Met-Thr-Trp-Met-Glu-Trp-Asp-Arg-Glu-Ile-Asn-Z-X,

wherein Y, X and Z have been defined previously.

The next peptide of interest, XII (23), will be encoded by the region extending from about bp 7708 through bp 7779. This peptide will include any oligopeptides coding for linear epitopes within the following amino acid sequence:

(XII) (23)

Y-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Z-X,

wherein X, Y and Z have been defined previously.

The next peptide of interest, XIII (79), will be encoded by the region extending from about bp 7543 through bp 7593. This peptide will include any oligopeptides coding for linear epitopes within the following amino acid sequence:

(XIII) (79)

Y-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-X,

wherein X and Y have been previously defined.

The next peptide of interest, XIIIa (78), will be encod d by the region xt nding from about bp 7561 through bp 7593. This peptide will include any oligopeptide coding for linear epitopes within the following amino acid sequence:

(XIIIa) (78)

Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys.

Of particular interest is the use of the mercaptan group of cysteines or thioglycolic acids used for acylating terminal amino groups or the like for linking two of the peptides or oligopeptides or combinations thereof by a disulfide linkage or a longer linkage. To achieve this, compounds may be employed having bis-haloacetyl groups, nitroarylhalides, or the like, where the reagents are specific for thio groups. Thus, the linking between the two mercapto groups of the different peptides or oligopeptides may be a single bond or a linking group of at least two, usually at least four, and not more than about 16, usually not more than about 14 carbon atoms. Of particular interest, is where a member of a sequence from the gag region is linked to a member from the env region. These chimeric peptides which may include non-amino acid linkages, may be further modified as will be described for the peptides and oligopeptides.

The subject peptides may be employed linked to a soluble macromolecular (e.g., ≥5kDal) carrier. Conveniently, the carrier may be a poly(amino acid), either naturally occurring or synthetic, to which antibodies are unlikely to be encountered in human serum. Illustrative polypeptides include poly-L-lysine, bovine serum albumin, keyhole limpet hemocyanin, bovine gamma globulin, etc. The choice is primarily one of convenience and availability.

With such conjugates, there will be at least one molecule of at least one subject peptide per macromolecule and not more than about 1 per 0.5kDal, usually not more than about 1 per 2kDal of the macromolecule. One or more different peptides may be linked to the same macromolecule.

Th manner of linking is conv ntional, employing such reagents as p-maleimidobenzoic acid,

p-methyldithiobenzoic acid, maleic acid anhydrid , succinic acid anhydride, glutaraldehyde, etc. The linkage may occur at the N-terminus, C-terminus or at a site intermediate the ends of the molecule. The subject peptide may be derivatized for linking, may be linked while bound to a support, or the like.

The compounds may be employed as labeled or unlabeled compounds depending upon their use. (By label is intended a molecule which provides, directly or indirectly, a detectable signal.) Various labels may be employed, such as radionuclides, enzymes, fluorescers, chemiluminescers, enzyme substrates, cofactors or inhibitors, particles, e.g., magnetic particles, combinations of ligands and receptors, e.g., biotin and avidin, or the like. In addition, the peptides may be modified in a variety of ways for binding to a surface, e.g., microtiter plate, glass beads, chromatographic surface, e.g., paper, cellulose, silica gel, or the like. The particular manner in which the polypeptides are joined to another compound or surface is conventional and finds ample illustration in the literature. See, for example, U.S. Patent Nos. 4,371,515; 4,487,715; and patents cited therein.

Various assay protocols may be employed for detecting the presence of either antibodies to retroviral proteins or retroviral proteins themselves. Of particular interest is using the peptide as the labeled reagent, where the label allows for a detectable signal, or binding the peptide, either directly or indirectly to a surface, where antibody to the peptide in the sample will become bound to the peptide on the surface. The presence of human antibody bound to the peptide can then be detected by employing a xenogeneic antibody specific for human immunoglobulin, normally both human IgM and IgG, or a label d prot in specific for immune complexes, .g., Rf factor or S. aureus protein A.

Various heterogeneous protocols may be employed, eith r competitive or non-competitive. Peptide may be bound to a surface or support ("support") and labeled antibody allowed to compete with antibody in the sample for the limited amount of bound peptide. The amount of label bound to the support would be related to the amount of competitive antibody in the sample.

Antibody could be bound to the support and the sample combined with labeled peptide. After contact of the reaction mixture with the bound antibody, the amount of label bound to the support would relate to the amount of cognate antibody in the sample.

Xenogeneic anti-human antibody, e.g., antibodies to the F_c of IgG and IgM (immunoglobulins), could be bound to a support. The sample would be contacted with the immunoglobulins and labeled peptide, whereby the amount of labeled peptide bound to the support would be indicative of the presence of the cognate antibodies.

Alternatively, homogeneous assays can be employed where the peptide is bound to an enzyme, fluorescer, or other label, where the binding of antibody to the peptide results in being able to discriminate between the label involved with a specific binding pair complex and label which is not involved in the complex. For assays involving such techniques, see for example U.S. Patent Nos. 3,817,837; 3,850,752; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876, whose disclosures are incorporated herein by reference.

As an illustration of the subject invention the subject peptides may be conjugated to a fluorescent molecule, such as fluorescein, rhodamine or umbelliferone. Various techniqu s may be used for d tecting complex formation with antibodies, e.g., fluorescence polarization. In this assay the

fluorescence polarization is different between complexed and uncomplexed peptide conjugate. Apparatuses are available for measuring changes in fluorescence polarization, e.g., TDx supplied by Abbott Laboratories, Chicago, IL.

Illustrative of an assay technique is the use of sample containers, e.g., microtiter plate wells, where the subject polypeptides or conjugates thereof are adhered to the container bottom and/or walls either covalently or non-covalently. The sample, normally human blood or serum diluted in an appropriately buffered medium, is added to the container and a sufficient time allowed for complex formation between the polypeptide(s) and any cognate antibodies in the sample. The supernatant is removed and the container washed to remove non-specifically bound proteins.

A labeled specific binding protein which specifically binds to the complex is employed for detection. To the container may be added xenogeneic antisera to human immunoglobulin, particularly anti-(human IgM and IgG) in an appropriately buffered medium. The xenogeneic antisera will normally be labeled with a detectable label, e.g., radionuclide or enzyme. Instead of antisera, proteins specific for the immune complex may be employed, e.g., S. aureus protein A. The label may then be detected. For example, with an enzyme, after removal of non-specifically bound enzyme label, a developer solution is added. The developer solution will contain an enzyme substrate and possibly enzyme cofactors, chromogens, etc., which, upon reaction, provide a colored or fluorescent product which may be detected colorimetrically or fluorimetrically, respectively.

The peptides can be prepared in a wide variety of ways. The peptid s, because of their relatively short size, may be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are

commercially available today and can be used in accordance with known protocols. See, for example, Stewart and Young, Solid Phase Peptide Synthesis, 2nd ed., Pierce Chemical Co., 1984; and Tam et al., J. Am. Chem. Soc. (1983) 105:6442.

Alternatively, hybrid DNA technology may be employed where a synthetic gene may be prepared by employing single strands which code for the polypeptide or substantially complementary strands thereof, where the single strands overlap and can be brought together in an annealing medium so as to hybridize. The hybridized strands may then be ligated to form the complete gene and by choice of appropriate termini, the gene may be inserted into expression vectors, which are readily available today. See, for example, Maniatis et al., Molecular Cloning, A Laboratory Manual, CSH, Cold Spring Harbor Laboratory, 1982. Or, the region of the viral genome coding for the peptide may be cloned by conventional recombinant DNA techniques and expressed (see Maniatis, supra).

DNA coding sequences which may be used for expressing peptides I - XIII are:

I (15) (TAT)GATTGTAAGACTATTTTAAAAGCATTGGGACCAG
CAGCTACACTAGAAGAAATGATGACAGCATGT

II (17) (TGT)TTAAAAGAGACCATCAATGAGGAAGCTGCAGAAT
GGGATAGAGTGCATCCAGTGCATGCA

III (92) GATAGAGTGCATCCAGTGCATGCAGGGCCTATTGCACCA
GGCCAG

IV (90) TATAGCCCTACCAGCATTCTGGACATAAGACAAGGACCA
AAAGAACCCTTTAGAGACTATGTAGACCGGTTCTATAAA
ACTCTAAGA

V (88) AATTGGATGACAGAAACCTTGTTGGTCCAAAATGCGAAC
CCAGATTGTAAGACTATTTTAAAAGCATTGGGACCAGCA
GCTACACTAGAAGAAATGATGACAGCATGT

VI (97) AGGGAGCTAGAACGATTCGCTGTTAATCCTGGCCTGTTA
GAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCTA
CAACCATCCCTTCAGACA

VII (71) GACACAGGACACAGCAGCCAGGTCAGCCAAAATTAC

VIII (36) GTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCA
AAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCA(TGT)

IX (56) ATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGA
TACCTAAAGGATCAACAG(TGT)

X (39) AGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAG
CTCCTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGC

XI (40) (TGT)AAATCTCTGGAACAGATTTGGAATAACATGACCT
GGATGGAGTGGGACAGAGAAATTAAC(TGT)

XII (23) (TGT)CATTCCTTAATTGAAGAATCGCAAAACCAGCAAG
AAAAGAATGAACAAGAATTATTGGAATTAGATAAATGG
(GGA)

XIII (79) AAGGATCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGA
AAACTCATTTGC

Fragments from these sequences may be employed for expression of peptide fragments, conservative base changes can be made, where the modified codon(s) code for the same amino acid(s), or non-conservative changes in the coding sequenc may be made, where the resulting amino acid may be a conservative or non-conservative change.

The coding sequence may be extended at either the 5'- or 3'-terminus or both termini to extend the

peptide, while retaining its epitopic site. The extension may provide for an arm for linking, e.g., to a label, such as an enzyme, for joining two or all of the peptides together in the same chain, for providing antigenic activity, or the like.

For expression, the coding sequence will be provided with start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in a cellular host, e.g., prokaryotic or eukaryotic, bacterial, yeast, mammal, etc.

The sequences by themselves, fragments thereof, or larger sequences, usually at least 15 bases, preferably at least 18 bases, may be used as probes for detection of retroviral RNA or proviral DNA. Numerous techniques are described, such as the Grunstein-Hogness technique, Southern technique, Northern technique, dot-blot, improvements thereon, as well as other methodology. See, for example, WO 83/02277 and Berent et al., Biotechniques (1985) 3:208.

Conveniently, the polypeptides may be prepared as fused proteins, where the polypeptide may be the N- or C-terminus of the fused polypeptide. The resulting fused protein could be used directly by itself as the reagent or the subject polypeptide may be cleaved from all or a portion of the remaining sequence of the fused protein. With a polypeptide where there are no internal methionines, by introducing a methionine at the fusion site, the polypeptide may be cleaved employing cyanogen bromide. Where there is an internal methionine, it would be necessary to provide for a proteolytic cleavage site, e.g., poly-lysine and/or -arginine or combinations thereof, or the internal m thionine could be substituted with an amino acid such as l ucine and an N-terminal methionine added for cyanogen bromide cleavag . A wide variety of proteases, including dipeptidases, are well known and

the appropriate processing signal could be introduced at the proper site. The processing signal may have tandem repeats so as to insure cleavage, since the presence of one or more extraneous amino acids will not interfere with the utility of the subject polypeptides.

Depending upon the nature of the assay, the physiological sample, e.g., saliva, blood, plasma, or serum, may be pretreated by dilution into an assay medium, which will usually be an aqueous buffered medium employing one of a variety of buffers, such as phosphate, tris, or the like. A preferred diluent is blotto (5% w/v nonfat dry milk, .01% thimerosal, .01% Antifoam A in .01 M sodium phosphate, pH 7.2, and .15 M NaCl). Usually the pH will be in the range of about 6 to 9. The sample will then be combined with the reagent in accordance with the appropriate protocol and sufficient time allowed for binding. Where a heterogeneous system is used, usually the stages will be followed by washes, to minimize non-specific binding. At the end of the procedure, the label will be detected in accordance with conventional ways.

Besides the use of the subject peptides and their analogs in assays, the subject peptides may also find use by themselves or in combination in vaccines. The peptides may be formulated in a convenient manner, generally at concentrations in the range of 1μg to 20mg/kg of host. Physiologically acceptable media may be used as carriers, such as sterile water, saline, phosphate buffered saline, and the like. Adjuvants may be employed, such as aluminum hydroxide gel, or the like. Administration may be by injection, e.g., intramuscularly, peritoneally, subcutaneously, intravenously, etc. Administration may be one or a plurality of times, usually at on to four we k intervals.

The following examples ar offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Peptides 15, 71, 88, 90, 92 and 97 w re assembled on a t-butyloxycarbonyl (BOC)-methylbenzyl-cysteine-phenyl-acetamidomethyl (PAM) polystyrene/divinylbenzene resin (Applied Biosystems, Inc., Foster City, CA). For carboxamide peptides 78 and 79 p-methylbenzhydrylamine polystyrene/divinylbenzene was used. Symmetrical anhydride couplings were carried out in an Applied Biosystems 430A synthesizer, except that glutamine and asparagine were coupled as hydroxybenzotriazole esters. Benzyl based side chain protection and BOC alpha-amine protection were used. Tryptophan was protected by the formyl moiety, methionine was protected by its sulfoxide, and dinitrophenol was used for protecting histidine. Protecting groups were removed by conventional procedures.

Peptide 36 was assembled on a benzhydrylamine polystyrene/divinylbenzene resin in a Beckman 990 peptide synthesizer (Beckman Instruments, La Brea, CA). Benzyl based side chain protection and BOC alpha-amine protection were used. All the residues were added by the direct dicyclohexylcarbodiimide method, except for glutamine which was coupled as the hydroxybenzotriazole ester.

Peptide 39 was synthesized on a benzhydrylamine resin as described for peptide 36 with asparagine also being coupled as the ester.

When the peptides were radiolabeled, it was by acetylating the amino terminus with ^{3}H-acetic acid and an excess of dicyclohexylcarbodiimide.

The peptides were deprotected and cleaved from the resin by the Tam "low-high" HF protocol (Tam et al., supra). Peptides 36, 39, 79, 78, 88, 90, 92 and 97 were extracted from the resin in 5% acetic acid and subjected to gel filtration chromatography in 5% acetic acid. Peptides 15 and 71 w re extracted in 0.5M ammonium carbonate/0.001M dithiothreitol (DTT) and

chromatographed in 0.05M ammonium carbonate/0.005M β-mercaptoethanol. Fractions containing the peptide were pooled and lyophilized. The integrity of the synthetic products was assured by ninhydrin monitoring after each coupling and by analytical reverse phase chromatography and amino acid analysis.

Peptides 90, 92 and 97 were polymerized by oxidation of their sulfhydryls to intermolecular disulfides. Briefly, the lyophilized reduced peptide was dissolved in minimal 6M guanidine HCl/0.1M sodium phosphate, pH 9.0, and allowed to oxidize overnight at room temperature.

Peptides 15, 23, 36, 40, 49, 50 and 56 synthesized above were conjugated to bovine serum albumin (BSA) which had been derivatized with N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), essentially as described by Ishikawa et al., J. of Immunoassay (1983) 4:209.

To 2ml of a BSA solution (20mg/ml in 0.1M potassium phosphate, pH 7.0) at 30°C was added 1.5ml of an SMCC solution (8mg/ml in dimethylformamide). The mixture was stirred magnetically for 1hr, after which it was centrifuged to remove any precipitated albumin. The clarified mixture was then subjected to gel filtration on Sephadex G-25 equilibrated in 0.1M potassium phosphate, pH 6.0. The protein-containing fractions, as determined by their absorbance at 280nm, were pooled and stored frozen at -70°C until needed.

The peptides synthesized above were dissolved in 0.1M sodium phosphate, pH 8.0 to a concentration of 5mg/ml (peptide 36), 8mg/ml (peptide 15) or 1.6mg/ml (peptide 39). To 1.5ml of each solution was added 2mg of solid DTT. The solutions were stirred for 30min at 30°C, after which they were subjected to gel filtration chromatography on Sephadex G-10, equilibrated in 0.1M potassium phosphate, pH 6.0. The tritium-containing fractions, as determined by scintillography of aliquots, were pooled and mixed with 1ml (0.5ml for

peptide V) of SMCC-derivatized BSA. The resultant mixtures were stirred at 30°C for 12hr and then dialyzed exhaustively against water.

The other peptides were prepared in accordance with the procedures described above and conjugated to BSA in accordance with the above described procedures. The ratio of peptide to BSA was determined by employing radiotracers in accordance with conventional ways.

	mols peptide / mol BSA
I (15)	14
II (17)	5
VIII (36)	9
IX (56)	17
X (39)	6
XI (40)	18
XII (23)	30 *

* may be erroneous and could be as high as 55.

Analysis by ELISA

The lyophilized peptide or protein/peptide conjugate was dissolved in 6M guanidine HCl. The guanidine solutions were diluted in 0.05M carbonate/bicarbonate buffer (pH 9.6) to a final peptide concentration of 8 to 40µg/ml just prior to plating in the 96-well plates. Fifty µl of peptide solution were aliquoted per microtiter well and incubated at 4°C overnight. Plates were then blocked with BLOTTO (5% [w/v] nonfat dry milk/0.01% thimerosal/0.01% antifoam A in 0.01M sodium phosphate, pH 7.2/0.15M sodium chloride) for one hour at 37°C. Sera were diluted 1:100 with a 1:1 mixture of BLOTTO and PBS (0.01M sodium phosphate, pH 7.3/0.15M NaCl), and 50µl of diluted sera was added to each well and incubated for one hour at 37°C. Th s ra were removed

and the plates wer wash d three times in wash buffer (0.15M NaCl/0.05% [w/v] Tween 20) before adding 100µl of the goat anti-human IgG/horseradish peroxidase conjugate (50% stock diluted 1:10,000 in 50mM sodium citrate/0.05% Tween 20/1% heat-inactivated normal goat serum; obtained from Antibodies, Inc., Davis, CA) for one hour at 37°C. The conjugate was removed and the plates washed three times with 0.15M NaCl/0.05% (w/v) Tween 20. The ELISA was developed by adding 100µl per well of substrate solution (10mg 3,3',5,5'-tetramethylbenzidine in 50ml 0.05M sodium citrate, pH 7.0) for 30min at room temperature. Reactions were stopped with 100µl per well of 3N H_2SO_4, and the optical density at 450nm determined by an automated ELISA reader.

Summary of Table 1

Table 1 gives ELISA results for all petpides that are immunoreactive.

Peptides 49 and 50 are part of peptide 36.

Peptide 56 partially overlaps peptide 39.

Peptide 49-BSA reactive with 10/10 positve sera; not reactive with 2/2 negative sera.

Peptide 50-BSA reactive with 10/10 positive sera; not reactive with 2/2 negative sera.

Peptide 56-BSA reactive with 10/10 positive sera; not reactive wtih 2/2 negative sera.

Peptide 40-BSA reactive with 10/10 positive sera; not reactive with 2/2 negative sera.

Peptide 23-BSA reactive with 10/10 positive sera; not reactive with 2/2 negative sera.

Peptide 15-BSA reactive with 10/10 positive sera; not reactive with 2/2 negative sera.

Peptide 36-BSA reactive with 9/10 positive sera; not r active with 2/2 negative sera.

In a larger panel, peptide 56 which partially overlaps peptide 39, is not reactive with all sera that are reactive with peptide 39. This suggests that there

are at 1 ast two reactive epitopes within peptide 39 or that peptides 39 and 56 contain non overlapping reactive epitopes.

Peptide 23 (both conjugated to BSA and unconjugated) was further tested against a larger panel of sera (23 positives, 8 negatives) and displays a sensitivity of 80-90%.

Summary of Table 2

Table 2 shows that two of the peptides derived from the gag region (#15 and #17) are reactive with LAV seropositive sera that are poorly reactive or unreactive with peptide 39. This supports the use of a combination of gag and env peptides to produce a more sensitive assay.

Summary of Table 3

Table 3 compares results obtained with peptides 15-BSA and 39 with results obtained with these peptides physically mixed (15-BSA + 39) or chemically combined (thiol-oxidized 15 + 39).

The result obtained when positive samples are assayed with either the physical or chemical combination of peptides 15 and 39 is generally higher than that obtained with either peptide alone. This is clearly demonstrated with samples 126, 131, 135, 138 and 1296.

Summary of Table 4

Table 4 compares results obtained with peptides 71, 78, 79, 88, 90, 92 and 97 in an ELISA assay. All of the peptides except one provide better than 70% correlation for positives and two peptides had 100% corr lation.

TABLE 1: COMPARISON OF PEPTIDES WITH A WHOLE VIRUS LYSATE IN AN ELISA ASSAY FOR THE DETECTION OF ANTIBODIES TO LAV

Positive Sera	Diagnosis	ELISA Using Whole Virus Lysate[1]	Confirmed as Seropositive[2]	BSA-Pep15	BSA-Pep36	BSA-Pep49	BSA-Pep50	Pep39	BSA-Pep56	BSA-Pep40	BSA-Pep23	Pep 23
155	LAS and/or homosexual	1.069	yes	0.525	1.679	0.955	1.678	1.167	1.675	0.603	1.640	0.111
124	LAS and/or homosexual	1.189	yes	1.329	1.465	1.334	2.207	1.073	1.842	1.462	2.117	2.127
138	LAS and/or homosexual	1.302	yes	0.378	0.159	0.204	0.374	0.514	0.643	0.774	0.960	0.106
133	LAS and/or homosexual	1.250	yes	0.365	0.567	0.409	0.581	1.036	0.627	1.297	2.077	N.D.
131	LAS and/or homosexual	1.220	yes	0.411	0.272	0.225	0.595	0.448	1.679	1.209	1.621	0.949
134	LAS and/or homosexual	1.050	yes	0.559	0.712	0.729	0.293	1.619	2.170	0.567	1.705	1.552
153	LAS and/or homosexual	2.000	yes	0.467	0.548	1.011	0.591	1.314	1.324	0.734	0.970	0.524
157	LAS and/or homosexual	1.349	yes	0.366	0.321	0.148	0.427	1.326	2.179	1.153	2.017	1.158
Y-1/CDC	LAS and/or homosexual	2.000	yes	2.109	1.022	1.547	1.928	1.305	2.115	1.257	1.565	0.762
501	LAS and/or homosexual	1.109	yes	2.374	1.168	1.938	2.209	1.167	1.170	0.625	0.467	0.059
1892	Healthy heterosexual	n.d.	n.d.[4]	0.128	0.113	0.119	0.124	0.045	0.143	0.141	0.253	0.034
639	Healthy heterosexual	0.123	not seropositive	0.159	0.142	0.102	0.186	0.038	0.355	0.251	0.286	0.024

TABLE 2: COMPARISON OF GAG PEPTIDES WITH PEPTIDE 39 IN AN ELISA ASSAY FOR THE DETECTION OF ANTIBODIES TO LAV

Serum No.	Diagnosis	Whole Virus Lysate[1]	Confirmed as Seropositive[2]	17-BSA	15-BSA	39
1296	Blood Donor	2.01	yes	0.633	0.65	0.11
501	Unknown	1.109	yes	0.18	2.04	2.15
129	LAS and/or homosexual	1.08	yes	0.62	0.49	0.42
154	LAS and/or homosexual	1.41	yes	0.26	0.26	0.35
. 7	LAS and/or homosexual	2.00	yes	0.79	1.02	0.22
641	Healthy heterosexual	0.20	n.d.	0.22	0.19	0.04
639	Healthy heterosexual	0.12	n.d.	0.20	0.16	0.05

TABLE 3: COMPARISON OF PEPTIDES 15 and 39 INDIVIDUALLY WITH PEPTIDES 15 AND 39 PHYSICALLY OR CHEMICALLY COMBINED IN AN ELISA ASSAY FOR THE DETECTION OF ANTIBODIES TO LAV

Serum No.	Diagnosis	Whole Virus Lysate[1]	Confirmed as Seropositive[2]	15-BSA	39	15-BSA 39	Thiol-oxidized 15 + 39
133	LAS and/or homosexual	1.250	yes	0.13	1.02	>2	1.88
134	LAS and/or homosexual	1.050	yes	0.21	1.62	>2	2.27
135	LAS and/or homosexual	1.310	yes	0.25	0.32	1.93	1.48
138	LAS and/or homosexual	1.302	yes	0.13	0.51	1.65	0.91
153	LAS and/or homosexual	2.000	yes	0.16	1.32	n.d.	1.89
154	LAS and/or homosexual	1.41	yes	0.19	0.35	n.d.	1.35
155	LAS and/or homosexual	1.069	yes	0.29	1.17	n.d.	1.83
157	LAS and/or homosexual	1.349	yes	0.14	1.33	n.d.	n.d..
666	Unknown	2.000	yes	1.60	1.39	>2	2.01
1296	Blood Donor	2,00	yes	0.65	0.11	0.99	0.16
633	Healthy heterosexual	0.222	not sero-positive	0.09	0.05	n.d.	n.d.
637	Healthy heterbsexual	0.097	not sero-positive	0.13	0.04	0.42	n.d.
639	Healthy heterosexual	0.123	not sero-positive	0.12	0.04	0.22	0.11
641	Healthy heterosexual	0.199	not sero-positive	0.18	0.03	0.49	0.13

TABLE 3: Continued. . .

Serum No.	Diagnosis	Whole Virus Lysate[1]	Confirmed as Seropositive[2]	15-BSA	19	15-BSA Thiol-oxidized 19	15-BSA Thiol-oxidized 15 + 19
501	Positive control	1.109	yes	1.39	1.17	>2.0	1.77
Y-1 CDC	Positive control pool	2.000	yes	1.02	1.30	>2.0	2.02
120	LAS[3] and/or homosexual	1.540	yes	0.19	1.37	n.d.	n.d.
121	LAS and/or homosexual	1.483	yes	0.09	1.51	>2.0	1.96
122	LAS and/or homosexual	1.283	yes	0.14	1.88	>2.0	2.33
124	LAS and/or homosexual	1.189	yes	0.60	1.06	n.d.	n.d.
125	LAS and/or homosexual	1.232	yes	0.18	1.53	n.d.	n.d.
126	LAS and/or homosexual	1.233	yes	0.24	0.51	>2	1.5
127	LAS and/or homosexual	1.046	yes	0.25	1.52	n.d.	n.d.
128	LAS and/or homosexual	1.284	yes	0.09	1.07	n.d.	n.d.
129	LAS and/or homosexual	1.081	yes	0.33	0.42	n.d.	n.d.
130	LAS and/or homosexual	0.912	yes	0.28	1.17	n.d.	n.d.
131	LAS and/or homosexual	1.220	yes	0.14	0.45	>2	1.22
132	LAS and/or homosexual	1.237	yes	0.15	1.24	>2	1.91

TABLE 3: Continued. . .

Serum No.	Diagnosis	Whole Virus Lysate[1]	Confirmed as Seropositive[2]	15-BSA	39	15-BSA 39	Thiol-oxidized 15 + 39
667	Healthy hetersexual	0.095	n.d.	0.15	0.04	0.42	n.d.
1890	Healthy heterosexual	n.d.	n.d.	0.15	0.05	0.39	0.15
1891	Healthy heterosexual	n.d.	n.d.	0.17	0.05	0.31	0.12
1892	Healthy heterosexual	n.d.	n.d.	0.08	0.05	0.18	0.07

TABLE 4: COMPARISON OF PEPTIDES 92, 90, 88, 97, 71, 79 and 78 IN AN ELISA ASSAY FOR THE DETECTION OF ANTIBODIES TO LAV

Serum No.	Confirmed as Seropositive	92	90	88	97	71	79	78
127	yes	.201	1.256	1.610	2.558	.476	2.346	.520
130	yes	.220	.900	1.912	2.341	.350	1.808	.438
124	yes	.105	1.175	.372	2.302	.514	1.086	.092
125	yes	.126	1.386	1.798	.395	.416	2.266	.281
128	yes	.122	.882	.201	.377	.246	1.144	.123
134	yes	.131	1.159	.358	2.455	.535	1.316	.118
135	yes	.120	.644	.157	1.231	.292	.381	.119
153	yes	.138	1.150	.180	.780	.352	1.039	.146
154	yes	ND	.623	.256	.365	.210	ND	ND
155	yes	.108	.845	.058	1.984	.185	1.584	.105
157	yes	.118	.936	.942	1.620	.536	1.162	.146
120	yes	.159	1.031	.740	.221	.362	1.546	.239
121	yes	.157	1.284	1.776	.396	.307	2.084	.205
132	yes	.100	.909	.422	.399	.398	1.386	.192
138	yes	.086	.495	ND	1.201	.285	.312	.093
133	yes	.100	.739	.143	.526	.312	.597	.114
131	yes	.112	.841	.197	.742	.188	1.150	.101
501	yes	.472	1.098	2.058	2.253	.341	1.768	.216
129	yes	.091	ND	ND	ND	ND	.562	.085
Y1	yes	ND	ND	2.228	ND	ND	ND	ND
N3	no	.074	.603	.106	.162	.101	.224	.076
N12	no	.075	.617	.131	.174	.088	.174	.056
N4	no	.058	.519	.128	.190	.090	.172	.040
639	no	.082	.474	.092	.115	.121	.153	.059
641	no	.081	.369	.090	.155	.169	.140	.085
N13	no	.079	.455	.111	.120	.100	.226	.122
N14	no	.054	.560	.098	.151	.085	.162	.070
N16	no	.077	.521	.083	.122	.070	.183	.079
Cut ff		0.10	0.70	0.20	0.20	0.20	0.30	0.20
Fraction of Confirmed Seropositive Samples Detected as P sitive		14/18	15/18	13/18	18/18	16/18	18/18	6/18

FOOTNOTES TO TABLES 1-3

[1] Prepared as described in U.K. application Serial No. 83/24800, filed September 15, 1983.

[2] Radiolabeled LAV antigens were disrupted in RIPA buffer (Gilead et al., Nature (1976) 264:263) and then were reacted with human serum. The resultant immune complexes were separated by binding to a Staphylococcus aureus adsorbent (Kessler, J. Immunology (1975) 115:1617) followed by multiple washings. Immuneprecipitated antigens were analyzed by SDS polyacrylamide gel electrophoresis (Laemmli, Nature (1970) 227:680) followed by fluorography. Presence of either a p25 or gp43 band was considered necessary and sufficient to confirm a sample as seropositive.

[3] LAS = lymphadenopathy syndrome.

[4] N.D. = not determined.

It is evident from the foregoing results that by employing one or a combination of peptides of the subject invention, a sensitive accurate test for the presence of antibodies to AIDS is provided. The subject peptides can be used by themselves or in combination with a screening assay or confirmatory assay, where the complete lysate or complete antigens may be employed as an independent procedure. Furthermore, because of the specificities of the peptides, one would anticipate that the DNA sequences coding for the peptides would also find similar specificity in a DNA hybridization assay. Thus, the subject invention allows for the detection of patients who have been exposed to the retroviral etiologic agent of lymphadenopathy syndrome and/or AIDS.

Although the foregoing invention has been described in some d tail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications

may be practiced within the scope of th appended claims.

WHAT IS CLAIMED IS:

1. In a method for detecting the presence of LAV/HTLV-III virus or antibody to LAV/HTLV-III virus where a sample is combined with a composition having epitopic sites immunologically competitive with LAV/HTLV-III epitopic sites, whereby antibodies bind to such protein composition to form a specific binding pair complex and the amount of complex formation is determined, the improvement which comprises:

employing in the assay medium as a reagent a compostion, containing at least one peptide which has at least five amino acids and fewer than 50 amino acids and is encoded for by the coding region of LAV/HTLV-III from bp 900 to bp 1421 or bp 7210 to bp 7815.

2. A method according to Claim 1, wherein said coding region is from bp 1320 to bp 1388 or bp 7231 to bp 7794.

3. A method according to Claim 2, wherein said composition includes at least two peptides, one encoded in the region bp 1320 to bp 1385 and the other encoded in the region bp 7231 to bp 7794.

4. A method according to Claim 3, where each determination is made individually for the binding to each of the peptides.

5. In a method for detecting the presence of LAV/HTLV-III virus or antibody to LAV/HTLV-III virus where a sample is combined with a composition having epitopic sites immunologically competitive with LAV/HTLV-III epitopic sites, whereby antibodies bind to each such prot in composition to form at least one specific binding pair compl x and the amount of complex formation is determined, the improvement which comprises:

mploying in th assay medium as a r agent a composition, containing at least one peptide which has at least five amino acids in a sequence which comes within the sequence of at least one of the following peptide sequences:

(I) (15)

Y-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Met-Met-Thr-Ala-Cys-X

(II) (17)

Y-Leu-Lys-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg-Val-His-Pro-Val-His-Ala-Z-X

(III) (92)

Y-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile-Ala-Pro-Gly-Gln-X

(IV) (90)

Y-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-Asp-Ile-Arg-Gln-Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-Asp-Arg-Phe-Tyr-Lys-Thr-Leu-Arg-Z-X

(V) (88)

Y-Asn-Trp-Nor-Thr-Glu-Thr-Leu-Leu-Val-Gln-Asn-Ala-Asn-Pro-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Nor-Nor-Thr-Ala-Cys-X

(VI) (97)

Y-Arg-Glu-Leu-Glu-Arg-Phe-Ala-Val-Asn-Pro-Gly-Leu-Leu-Glu-Thr-Ser-Glu-Gly-Cys-Arg-Gln-Ile-Leu-Gly-Gln-Leu-Gln-Pro-Ser-L u-Gln-Thr-X

(VII) (71)

Y-Asp-Thr-Gly-His-Ser-Ser-Gln-Val-Ser-Gln-Asn-Tyr

(VIII) (36)

Val-Lys-Ile-Glu-Pro-Leu-Gly-Val-Ala-Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Ala-Z-X

(IX) (56)

Ile-Lys-Gln-Leu-Gln-Ala-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Z-X

(X) (39)

Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-X

(XI) (40)

Y-Lys-Ser-Leu-Glu-Gln-Ile-Trp-Asn-Asn-Met-Thr-Trp-Met-Glu-Trp-Asp-Arg-Glu-Ile-Asn-Z-X

(XII) (23)

Y-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Z-X

(XIII) (79)

Y-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-X,

where X is OH or NH_2, and Y and Z, when present, are amino acids added to facilitate coupling, and where said peptide is free of other peptides or conjugated to a macromol cule for which antibodi s in human sera are substantially absent.

6. A method according to Claim 5, wherein said composition includes at least two of said peptides, at least one of said peptides having the amino acid sequence according to peptide sequences I to VII and at least one of said peptides having an amino acid sequence according to peptides VIII to XIII.

7. A method according to Claim 6, wherein one of the peptides has one of the following sequences:

(VIIIa) (49)
Y-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-X

(VIIIb) (50)
Y-Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-X

(IXa) (56/39)
Y-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Z-X

(IXb) (39/56)
Y-Ile-Lys-Gln-Leu-Gln-Ala-Arg-Ile-Leu-Z-X

(XIIIa) (78)
Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys,

where X is OH or NH_2, and Y, when present, is an amino acid added to facilitate coupling.

8. A method according to Claim 1, wherein said composition includes at least one peptide selected from p25 and one from p18.

9. A method according to Claim 5, wherein said composition is bound to a solid surface.

10. A method for determining the presence of antibodies to LAV/HTLV-III in a physiological fluid, said method comprising:

introducing a human serum, plasma or blood sample into a sample container coated at least in part with at least one peptide having at least five amino acids which comes within the sequence of at least one of the following peptide sequences:

(I) (15)

Y-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Met-Met-Thr-Ala-Cys-X

(II) (17)

Y-Leu-Lys-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg-Val-His-Pro-Val-His-Ala-X

(III) (92)

Y-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile-Ala-Pro-Gly-Gln-X

(IV) (90)

Y-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-Asp-Ile-Arg-Gln-Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-Asp-Arg-Phe-Tyr-Lys-Thr-Leu-Arg-Z-X

(V) (88)

Y-Asn-Trp-Nor-Thr-Glu-Thr-Leu-Leu-Val-Gln-Asn-Ala-Asn-Pro-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Nor-Nor-Thr-Ala-Cys-X

(VI) (97)

Y-Arg-Glu-Leu-Glu-Arg-Phe-Ala-Val-Asn-Pro-Gly-Leu-Leu-Glu-Thr-Ser-Glu-Gly-Cys-Arg-Gln-Ile-Leu-Gly-Gln-Leu-Gln-Pro-Ser-Leu-Gln-Thr-X

(VII) (71)

Y-Asp-Thr-Gly-His-Ser-Ser-Gln-Val-Ser-Gln-Asn-Tyr

(VIII) (36)

Val-Lys-Ile-Glu-Pro-Leu-Gly-Val-Ala-Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Ala-Z-X

(IX) (56)

Ile-Lys-Gln-Leu-Gln-Ala-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Z-X

(X) (39)

Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-X

(XI) (40)

Y-Lys-Ser-Leu-Glu-Gln-Ile-Trp-Asn-Asn-Met-Thr-Trp-Met-Glu-Trp-Asp-Arg-Glu-Ile-Asn-Z-X

(XII) (23)

Y-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Z-X

(XIII) (79)

Y-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-X,

where X is OH or NH_2, and Y and Z, if present, are amino acids added to facilitate coupling, wherein said peptides are free of other peptides or conjugated to a macromolecule for which antibodies in human sera are substantially absent;

incubating for a sufficient time for complex formation to occur; and

determining the formation of complex by employing a labeled specific binding protein which binds to said complex and provides a detectable signal.

11. A method according to Claim 10, wherein said peptide is conjugated to a water soluble protein of at least 5kDal as said macromolecule.

12. A method according to Claim 10, wherein two of said peptides are covalently linked together through a bond or chain.

13. A method according to Claim 10, wherein said specific binding protein is antibody to human immunoglobulin.

14. A method according to Claim 10, wherein said composition includes at least two of said peptides, at least one peptide coming from peptides I to VII and at least one peptide coming from peptides VIII to XIII.

15. A method for determining the presence of antibodies to LAV/HTLV-III in a physiological fluid, said method comprising:

combining a human serum, plasma or blood sample with at least one labeled peptide having at least five amino acids in a sequence which comes within the sequence of at least one of the following peptid sequences:

(I) (15)

Y-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Met-Met-Thr-Ala-Cys-X

(II) (17)

Y-Leu-Lys-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg-Val-His-Pro-Val-His-Ala-X

(III) (92)

Y-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile-Ala-Pro-Gly-Gln-X

(IV) (90)

Y-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-Asp-Ile-Arg-Gln-Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-Asp-Arg-Phe-Tyr-Lys-Thr-Leu-Arg-Z-X

(V) (88)

Y-Asn-Trp-Nor-Thr-Glu-Thr-Leu-Leu-Val-Gln-Asn-Ala-Asn-Pro-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Nor-Nor-Thr-Ala-Cys-X

(VI) (97)

Y-Arg-Glu-Leu-Glu-Arg-Phe-Ala-Val-Asn-Pro-Gly-Leu-Leu-Glu-Thr-Ser-Glu-Gly-Cys-Arg-Gln-Ile-Leu-Gly-Gln-Leu-Gln-Pro-Ser-Leu-Gln-Thr-X

(VII) (71)

Y-Asp-Thr-Gly-His-Ser-Ser-Gln-Val-Ser-Gln-Asn-Tyr

(VIII) (36)

Val-Lys-Ile-Glu-Pro-Leu-Gly-Val-Ala-Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Ala-Z-X

(IX) (56)

Ile-Lys-Gln-Leu-Gln-Ala-Arg-Ile-Leu-
Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Z-X

(X) (39)

Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-
Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-
Ser-Gly-Lys-Leu-Ile-Cys-X

(XI) (40)

Y-Lys-Ser-Leu-Glu-Gln-Ile-Trp-Asn-Asn-
Met-Thr-Trp-Met-Glu-Trp-Asp-Arg-Glu-
Ile-Asn-Z-X

(XII) (23)

Y-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-
Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-
Glu-Leu-Asp-Lys-Trp-Z-X

(XIII) (79)

Y-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-
Cys-Ser-Gly-Lys-Leu-Ile-Cys-X,

where X is OH or NH_2, and Y and Z, if present, are amino acids added to facilitate coupling, and said peptides are conjugated to a protein for which antibodies in human sera are substantially absent or unconjugated to a protein;

incubating for a sufficient time for complex formation to occur; and

determining the formation of complex as a result of change in a detectable signal resulting from complex formation.

16. A method according to Claim 15, wherein said label is a fluorescer.

17. A method according to Claim 16, wherein complex formation is determined by fluorescence polarization.

18. A method according to Claim 15, wherein said label is an enzyme.

19. A method according to Claim 18, wherein complex formation is determined as a result of enzyme activity modulation.

20. A peptide of the formula:

(I) (15)

Y-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Met-Met-Thr-Ala-Cys-X,

where X is OH or NH_2, and Y, if present, is an amino acid added to facilitate coupling, N-terminal acetylated I, and I linked to a peptide or protein of at least 5,000 molecular weight, which peptide or protein does not normally bind to antibodies present in a human host.

21. A peptide of the formula:

(II) (17)

Y-Leu-Lys-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg-Val-His-Pro-Val-His-Ala-X,

where X is OH or NH_2, and Y, if present, is an amino acid added to facilitate coupling, N-terminal acetylated II, and II link d to a peptide or protein of at least 5,000 molecular weight, which peptid or protein does not normally bind to antibodies present in a human host.

22. A peptide of the formula:

(III) (92)

Y-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-
Ile-Ala-Pro-Gly-Gln-X,

where X is OH or NH_2, and Y, if present, is an amino acid added to facilitate coupling, N-terminal acetylated III, and III linked to a peptide or protein of at least 5,000 molecular weight, which peptide or protein does not normally bind to antibodies present in a human host.

23. A peptide of the formula:

(IV) (90)

Y-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-Asp-Ile-Arg-
Gln-Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-
Asp-Arg-Phe-Tyr-Lys-Thr-Leu-Arg-Z-X,

where X is OH or NH_2, and Y, if present, is an amino acid added to facilitate coupling, N-terminal acetylated IV, and IV linked to a peptide or protein of at least 5,000 molecular weight, which peptide or protein does not normally bind to antibodies present in a human host.

24. A peptide of the formula:

(V) (88)

Y-Asn-Trp-Nor-Thr-Glu-Thr-Leu-Leu-Val-Gln-
Asn-Ala-Asn-Pro-Asp-Cys-Lys-Thr-Ile-Leu-Lys-
Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Nor-
Nor-Thr-Ala-Cys-X,

where X is OH or NH_2, and Y, if present, is an amino acid added to facilitate coupling, N-terminal acetylated V, and V link d to a peptide or prot in of

at least 5,000 molecular w ight, which peptide or protein does not normally bind to antibodies present in a human host.

25. A peptide of the formula:

(VI) (97)
Y-Arg-Glu-Leu-Glu-Arg-Phe-Ala-Val-Asn-Pro-Gly-Leu-Leu-Glu-Thr-Ser-Glu-Gly-Cys-Arg-Gln-Ile-Leu-Gly-Gln-Leu-Gln-Pro-Ser-Leu-Gln-Thr-X,

where X is OH or NH_2, and Y, if present, is an amino acid added to facilitate coupling, N-terminal acetylated VI, and VI linked to a peptide or protein of at least 5,000 molecular weight, which peptide or protein does not normally bind to antibodies present in a human host.

26. A peptide of the formula:

(VII) (71)
Y-Asp-Thr-Gly-His-Ser-Ser-Gln-Val-Ser-Gln-Asn-Tyr

where Y, if present, is an amino acid added to facilitate coupling, N-terminal acetylated VII, and VII linked to a peptide or protein of at least 5,000 molecular weight, which peptide or protein does not normally bind to antibodies present in a human host.

27. A peptide of the formula:

(VIII) (36)
Val-Lys-Ile-Glu-Pro-Leu-Gly-Val-Ala-Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Ala-Z-X,

where X is OH or NH_2, and Z, if present, is an amino acid added to facilitate coupling, N-t rminal acetylated VIII, and VIII linked to a peptide or protein of at least 5,000 molecular weight, which peptide or protein does not normally bind to antibodies present in a human host.

28. A peptide of the formula:

(IX) (56)

Ile-Lys-Gln-Leu-Gln-Ala-Arg-Ile-Leu-
Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Z-X,

where X is OH or NH_2, and Z, if present, is an amino acid added to facilitate coupling, N-terminal acetylated IX, and IX linked to a peptide or protein of at least 5,000 molecular weight, which peptide or protein does not normally bind to antibodies present in a human host.

29. A peptide of the formula:

(X) (39)

Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-
Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-
Ser-Gly-Lys-Leu-Ile-Cys-X,

where X is OH or NH_2, N-terminal acetylated X, and X linked to a peptide or protein of at least 5,000 molecular weight, which peptide or protein does not normally bind to antibodies present in a human host.

30. A peptide of the formula:

(XI) (40)

Y-Lys-Ser-L u-Glu-Gln-Ile-Trp-Asn-Asn-
Met-Thr-Trp-Met-Glu-Trp-Asp-Arg-Glu-
Ile-Asn-Z-X,

where X is OH or NH_2, and ach of Y and Z, if present, is an amino acid added to facilitate coupling, N-terminal acetylated XI, and XI linked to a peptide or protein of at least 5,000 molecular weight, which peptide or protein does not normally bind to antibodies present in a human host.

31. A peptide of the formula:

(XII) (23)

Y-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Z-X,

where X is OH or NH_2, and each of Y and Z, if present, is an amino acid added to facilitate coupling, N-terminal acetylated XII, and XII linked to a peptide or protein of at least 5,000 molecular weight, which peptide or protein does not normally bind to antibodies present in a human host.

32. A peptide of the formula:

(XIII) (79)

Y-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-X,

where X is OH or NH_2, and each of Y and Z, if present, is an amino acid added to facilitate coupling, N-terminal acetylated XIII, and XIII linked to a peptide or protein of at least 5,000 molecular weight, which peptide or protein does not normally bind to antibodies present in a human host.

33. A vaccine composition comprising at least one peptide of the peptides:

(I) (15)

Y-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Met-Met-Thr-Ala-Cys-X

(II) (17)

Y-Leu-Lys-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg-Val-His-Pro-Val-His-Ala-Z-X

(III) (92)

Y-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile-Ala-Pro-Gly-Gln-X

(IV) (90)

Y-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-Asp-Ile-Arg-Gln-Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-Asp-Arg-Phe-Tyr-Lys-Thr-Leu-Arg-Z-X

(V) (88)

Y-Asn-Trp-Nor-Thr-Glu-Thr-Leu-Leu-Val-Gln-Asn-Ala-Asn-Pro-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Nor-Nor-Thr-Ala-Cys-X

(VI) (97)

Arg-Glu-Leu-Glu-Arg-Phe-Ala-Val-Asn-Pro-Gly-Leu-Leu-Glu-Thr-Ser-Glu-Gly-Cys-Arg-Gln-Ile-Leu-Gly-Gln-Leu-Gln-Pro-Ser-Leu-Gln-Thr

(VII) (71)

Asp-Thr-Gly-His-Ser-Ser-Gln-Val-Ser-Gln-Asn-Tyr

(VIII) (36)

Val-Lys-Ile-Glu-Pro-Leu-Gly-Val-Ala-Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Ala-Z-X

(IX) (56)

Ile-Lys-Gln-Leu-Gln-Ala-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Z-X

(X) (39)

Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys-X

(XI) (40)

Y-Lys-Ser-Leu-Glu-Gln-Ile-Trp-Asn-Asn-Met-Thr-Trp-Met-Glu-Trp-Asp-Arg-Glu-Ile-Asn-Z-X

(XII) (23)

Y-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Z-X

(XIII) (79)

Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys,

where X is OH or NH_2, and each of Y and Z, when present, are amino acids added to facilitate coupling,

or said peptides conjugated to an immunogenic protein, said peptides or conjugates being present in an amount to provide an immunogenic response in a physiologically acceptable carrier.

34. A vaccine according to Claim 34, wherein said peptides are coupled to immunogenic proteins.

35. A vaccine according to Claim 34, wherein at least one peptide is I to VII and at least one peptide is VIII to XIII.

36. A vaccine according to any of Claims 33, 34 or 35, wherein said immunogenic protein is tetanus toxoid.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US86/00831

I. CLASSIFICATI N F SUBJECT MATTER (If several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl. 4 C12Q 1/70;G01N 33/50;C07K 7/06,08,10;A61K 39/00,12
U.S. Cl. 435/5,7;530/324,327;424/88,89

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	435/5,7 530/324,326,327; 424/88,89

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A,P	US, A, 4,520,113 Published 28 May 1985 Gallo,et al	1-19
A,P	Robey, et al, Science, Vol. 228, Published 03 May 1985, pages 593-595	20-36
A	Gallo, et al, Science, Vol. 228, Published 05 April 1985, pages 93-96	20-36
A,P	Chang, et al, Nature, Vol. 315, Published 09 May 1985, pages 151-154	1-36
A	Ratner, et al, Nature, Vol. 313, Published 24 January 1985, pages 277-284	20-32
A,P	Crowl, et al, Cell, Vol. 41, Published July 1985	1-36

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
01 July 1986	14 AUG 1986
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	Debbie A. Phillips

Form PCT/ISA/210 (second sheet) (October 1981)

FURTHER INF RMATI N C NTINUED FR M THE ECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers....., because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1-19 drawn to methods of detection of viruses, classified in U.S. class 435, subclass 5 and 7.

II. Claims 2-32 drawn to peptides and fragments, classified in U.S. class 530, subclass 324 and 327.

III. Claims 33-36 drawn to vaccines, classified in U.S. class 424, subclass 88 and 89.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.